

## **IN THE SPECIFICATION**

### **ABSTRACT**

Please amend the **Abstract** as follows:

The present invention provides a method of reducing the period within which a plant's natural defence mechanism responds to attack by a plant pathogen, the method comprising causing the plant to maintain, in at least a part of the plant, a level of BiP which is greater than the endogenous level for said plant in non-stressful conditions. Increased BiP levels can be achieved by transformation with a nucleic acid encoding BiP or calreticulin or modifying signal transduction pathways leading to BiP induction. BiP levels can be increased above endogenous levels by over-expressing BiP or calreticulin. The invention also provides for a modified plant produced by the method of the invention.

### **DETAILED DESCRIPTION OF THE INVENTION**

#### **Example 6**

Please amend the paragraph at page 17, line 33 through page 18, line 7 with the following amended paragraph.

To compare the CDE- and UPR-mediated induction of BiP, we wanted to test if both stimuli are additive. For this purpose, we prepared protoplasts which are known to exhibit induced levels of  $\beta$ -1,3-glucanase (Denecke et al., 1995). This is not surprising as protoplasts are prepared with CDEs. These protoplasts were then treated with tunicamycin, to superimpose the UPR onto the CDE response. Figure [[7]] 6 shows that both stimuli are additive, exhibited by a further induction of BiP by tunicamycin. This suggests that both mechanisms are different. Interestingly,  $\beta$ -1,3-glucanase expression is inhibited by tunicamycin. The additional ER stress could trap BiP in malformed protein complexes, thus making it unavailable to promote PR protein synthesis on the rough ER. The results suggested that although BiP induction alone is not sufficient to trigger PR protein synthesis, sufficient BiP levels are required to promote PR gene expression.

### **Example 11**

Please amend the paragraph at page 20, line 26 through page 21, line 8 with the following amended paragraph.

To gain further insight into the signal transduction pathways leading to the induction of BiP and PR1, an SA non-responsive mutant of *Arabidopsis thaliana* (*sail*) was used which does not express PR1 in the presence of SA (Shah et al, 1997). WT and *sail* mutants of the same ecotype were sprayed with SA (5 mM) and incubated in the light. Total RNA was extracted and probed with the PR1 and BiP gene from *Arabidopsis*. The induction of PR1 in WT *Arabidopsis* was detected 3 hours after SA-treatment and continued to increase until 8 hours (Figure [[10]] 11). BiP showed the same expression profile as seen in tobacco plants when treated with SA. As in tobacco plants, BiP mRNA levels increase prior to PR1 transcripts in SA treated *Arabidopsis* plants (Figure [[10]] 7) and diminish after prolonged incubations (data not shown). In the *Arabidopsis sail* mutant, the PR1 induction was completely abolished during SA-treatment as expected (Shah et al, 1997). In contrast, BiP mRNA levels in the mutants showed exactly the same induction pattern as in the wild-type plant. This demonstrates that either a different SA-dependent signal transduction pathway is used to induce the BiP gene, or that the regulatory protein which is defective in *sail* mutants is located downstream of the BiP gene in the signal transduction pathway leading from SA to induced PR1 or BiP gene induction.

### **Example 12**

Please amend the paragraph at page 21, lines 12-29 with the following amended paragraph.

To distinguish between the two possible working models, we tested PR1 gene expression in transgenic plants carrying the BiP coding region under the control of the strong constitutive Cauliflower Mosaic Virus (CaMV) 35S promoter. If BiP is simply located on the signal transduction pathway upstream of the *sail* mutation[[.]], BiP overproduction alone should lead to induced PR1 gene expression. Transgenic plants which show 5-fold increased BiP steady state protein levels and 142-fold increased BiP transcript levels were used to test basal PRI mRNA

levels and SA-mediated PR1 induction. As shown in Figure[[11]] 12A, basal BiP mRNA levels are much higher in the BiP overproducing plants. The weak induction by SA was unexpected but could be due to the influence of SA on the CaMV35S promoter itself (Qin et al, 1994). Figure [[11]] 12 shows that the overproduction of BiP alone does not replace the SA signal because it does not lead to induction of the PR1 gene (compare lanes 0 with each other). However, the BiP overproducing plants show a more rapid PR1 induction upon SA treatment compared to the wild-type plant. Together the results show that high BiP levels in the ER promote the SA-mediated PR1 induction but cannot replace the SA signal. This suggests that a branched signal transduction pathway leads to the induction of BiP and PR1 upon SA treatment and that there is cross-talk between the two branches of the pathway.

### **Example 13**

Please amend the paragraph at page 21, line 34 through page 22, line 11 with the following amended paragraph.

We have established a model system based on the comparison of protein biosynthesis in the cytosol and on the rough ER using transient expression. A plasmid was constructed (pNL200, Figure [[12A]] 13A) containing two genes, one encoding the secreted barley  $\alpha$ -amylase (Rogers, 1985) and the other encoding the cytosolic marker  $\beta$ -glucuronidase (GUS; Jefferson et al., 1987).  $\alpha$ -Amylase was used to measure secretory protein biosynthesis, and GUS was used to Control for transfection efficiency and overall cell viability. We compared cells under normal culture conditions with cells subjected to ER stress by treatment with tunicamycin. Figure [[12B]] 13B shows that tunicamycin does not affect cell viability during the course of the experiment, as monitored with the internal marker GUS, confirming previous results (Denecke et al., 1990). In contrast, total  $\alpha$ -amylase activity in the cell suspension was greatly reduced. Since  $\alpha$ -amylase is not glycosylated, tunicamycin should not have a direct effect on this protein. The tunicamycin effect is protein-specific and not dependent on the promoter used. Therefore, we postulated that during tunicamycin stress,  $\alpha$ -amylase synthesis, translocation, or folding is compromised.

**Example 14**

Please amend the paragraph at page 22, line 32 through page 23, line 5 with the following amended paragraph.

Experiments were conducted in such a manner that similar internal marker activities (GUS) were obtained in each experiment. The  $\alpha$ -amylase activity of the total extract was then correlated with the final GUS activities, and Figure [[13A]] 14A shows the ratio of  $\alpha$ -amylase activity to GUS activity. If PAT is coexpressed, tunicamycin leads to a reduction of  $\alpha$ -amylase activities, as shown in Figure [[12]] 14B. BiP coexpression alone leads to slightly lower  $\alpha$ -amylase activities compared to PAT coexpression, but no further reduction of  $\alpha$ -amylase activity was seen during tunicamycin treatment. Coexpression of the antisense construct was indistinguishable from PAT coexpression. Figure [[13B]] 14B shows the percentage of  $\alpha$ -amylase activity that remains after tunicamycin treatment and illustrates clearly that BiP overexpression protects the cells from tunicamycin stress.

**Example 15**

Please amend the paragraph at page 23, lines 11-19 with the following amended paragraph.

Figure [[14]] 15 shows that the transcript level of vacuolar PR protein  $\beta$ -1,3-glucanase rapidly decreases during UPR-induced BiP transcription but begins to rise again when the BiP mRNA level has reached its maximum. During prolonged incubation times, transcript levels encoding secretory proteins recover to almost normal (initial) values again. Identical results were obtained with other transcripts encoding the secretory proteins acidic chitinase and basic chitinase present in tobacco protoplasts, showing that the effect is not restricted to  $\beta$ -1,3-glucanase (data not shown). The data suggest that inhibition of secretory protein synthesis, observed during ER stress (Figure [[12]] 15), occurs prior to translation.

**Example 16**

Please amend the paragraph at page 23, lines 23-32, line 5 with the following amended paragraph.

Figure [[15A]] 16A shows that in BiP overexpressing tobacco protoplasts (89), no tunicamycin-mediated reduction of  $\beta$ -1,3-glucanase mRNA levels is observed, consistent with the hypothesis that the effect is due to limiting amounts of BiP. Overexpression of a BiP derivative lacking the ER retention signal (801) only partially restores the  $\beta$ -1,3-glucanase mRNA level under ER stress conditions (Figure~~[[15B]]~~ 16B). This would be expected, as the lack of a retention signal will result in a lower BiP level in the ER lumen. The result also suggests that it is the level of BiP in the ER lumen, and not the level of BiP transcripts which is important in this respect.